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The paraventricular nucleus and heart failure.

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## **NEW FINDINGS**

### **What is the topic of this review?**

This review gives an update on the cellular and molecular mechanisms within the autonomic nervous system involved in non-pathological and pathological cardiovascular regulation.

### **What advances does it highlight?**

For cardiovascular homeostasis in non-pathological conditions to be maintained discrete neural networks utilising specified signaling mechanisms at both cellular and molecular levels are required.

In heart failure, the cell signaling protein partners CAPON and PIN decrease the bioavailability of nitric oxide by inhibiting neuronal nitric oxide synthase leading to the removal of tonic neuronal inhibition. Following a myocardial infarction, proinflammatory cytokines in the paraventricular nucleus and the subsequent generation of reactive oxygen species, via angiotensin II activation of the angiotensin II type 1 receptor, increase neuronal excitability further leading to sympathetic excitation.

## **ABSTRACT**

A pathological feature of heart failure is abnormal control of the sympathetic nervous system. The paraventricular nucleus of the hypothalamus (PVN) is one of the most important central sites involved in regulating sympathetic tone and is, in part, responsible for the dysregulation of the sympathetic nervous system evident in heart failure. Generation of sympathetic tone in response to fluctuations in cardiovascular regulation uses discrete anatomical pathways and neurochemical modulators. Direct and indirect projections from the PVN pre-autonomic neurons innervate the sympathetic preganglionic neurons in the spinal cord, which in turn innervate sympathetic ganglia that give rise to the sympathetic nerves. Pre-autonomic neurons of the PVN themselves receive afferent input arising from the nucleus tractus solitarii, and viscerosensory receptors convey cardiovascular fluctuations to the nucleus tractus solitarii. The PVN contains excitatory and inhibitory interneurons, whose balance determines the sympathetic tone. In non-pathological conditions, the tonic inhibition of the PVN pre-autonomic neurons is mediated by GABA- and NO-releasing neurons. In heart failure, the pre-autonomic neurons are disinhibited by the actions of the excitatory neurotransmitters glutamate and angiotensin II, leading to increased sympathetic activity. A key feature of the disinhibition is a reduction in the bioavailability of NO as a consequence of disrupted CAPON and PIN signalling mechanisms within the neuron. Another critical feature that contributes to increased neuronal excitation within the PVN is the production of proinflammatory cytokines immediately following a myocardial infarction, the activation of the angiotensin II type 1 receptor and the production of reactive oxygen species. By examining the changes associated with the sympathetic nervous system pathway we will progress our understanding of sympathetic regulation in heart failure, identify gaps in our knowledge and suggest new therapeutic strategies.

## INTRODUCTION

Cardiovascular homeostasis is maintained by neurohumoral integration involving both the neuroendocrine and autonomic nervous systems (Guyenet, 2010). Processing of information by these two major systems using specific neuronal networks within the central nervous system (CNS) generates adaptive neurohumoral responses. Neurohumoral activation is also seen as a contributor to numerous diseases including hypertension and heart failure (HF). Heart failure can be characterised by neurohumoral activation in response to decreased cardiac output and under-perfusion of tissue (Watson et al., 2006). Initially in the short term these compensatory mechanisms are beneficial to maintaining homeostasis, however chronic activation leads to further deterioration in heart function and progression of the HF. Furthermore, the increased sympathetic nerve activity (SNA) inherent in HF is viewed as a major contributor to morbidity and mortality in HF patients (Warion et al., 2006). Therefore, understanding the precise anatomical pathways and cellular mechanisms generating the increased sympathetic outflow by the brain is important physiologically and clinically in HF.

### **The paraventricular nucleus (PVN) and heart failure**

The organization of the PVN and its role in fluid balance and vasopressin release make the PVN a pivotal central site responsible for mediating sympathetic outflow during normal and HF states (Pyner, 2009). Indeed, numerous studies have demonstrated that there is increased neuronal activation of the PVN in rats with HF (Li & Patel, 2003; Han et al., 2010; Xu et al., 2012) that translates at the level of the end-organ as increased renal and cardiac sympathetic nerve activity (May et al., 2010). Of the neurotransmitters localized to the PVN, glutamate and angiotensin (ANGII) exert excitatory effects on sympathetic outflow, whereas NO and GABA are inhibitory mediators of the outflow (Li & Patel, 2003). Much attention has been paid to how excitatory and inhibitory transmitter interactions in the PVN regulate sympathetic outflow. It is now accepted that a down regulation of the inhibitory input and an upregulation of the excitatory input onto the PVN pre-autonomic neurons play a significant role in sympathetic dysfunction in HF.

### **Central neuronal circuitry involved in sympathetic regulation**

The PVN, the rostral ventrolateral medulla (RVLM) and nucleus of the solitary tract (NTS) have been identified as critical sites within the CNS that regulate sympathetic activity (Guyenet, 2006,). The NTS provides the access to the CNS for information arising from the viscerosensory afferents. From this, the NTS then initiates adjustments in reflexes that maintain cardiovascular homeostasis using its connections between the medullary-RVLM and supramedullary-PVN sites (Pyner, 2009). The PVN and RVLM adjust sympathetic output in response to the information forwarded by the NTS to maintain cardiovascular homeostasis. The PVN and RVLM send mono- and polysynaptic projections to the sympathetic preganglionic neurons (SPNs) in the intermediolateral cell column of the spinal cord. Sympathetic preganglionic neurons are an integral part of the central circuitry and are the source of all sympathetic outflow to every organ in the body (Figure 1A; Pyner, 2009).

### **The paraventricular nucleus of the hypothalamus**

The PVN is a pivotal brain centre involved in producing co-ordinated neurohumoral responses (Swanson & Sawchenk, 1983). The PVN is a functionally heterogeneous nucleus comprised of diverse groups of neurons (Swanson & Sawchenko, 1983). The main groups are magnocellular, which synthesise vasopressin and oxytocin to be released from the posterior pituitary in response to a number of stimuli including hyperosmolarity. The parvocellular neuroendocrine neurons that secrete hypophysiotropic hormones and parvocellular preautonomic neurons that control sympathetic nerve activity. In total more than 30 neurotransmitters have been localized to neurons within the PVN (Stern 2004; Pyner 2009;)

### **Efferent projections from the PVN**

That the hypothalamus exerts an important regulatory influence on the autonomic nervous system has long been known (Bard, 1928). We now know the parvocellular neurons of the PVN are reciprocally connected to the NTS (Swanson and Sawchenko, 1983); directly project and terminate on or close to target specified SPNs; project and terminate close to spinally projecting RVLM neurons that themselves terminate

on the target specified SPNs; and send collaterals to both the RVLM and SPNs (Pyner 2009). There may be further pathways we have yet to identify. Activation of the PVN-RVLM and PVN-SPN pathways contribute to the changes in sympathetic nerve activity (SNA) observed after activation of the PVN and stimulation of the PVN can be correlated with renal SNA (Deering & Coote, 2000).

### **Afferent projections to the PVN**

Cardiovascular afferents relaying information about pressure, volume and oxygen saturation terminate mainly within the NTS (Coote, 2005) and signals from each of these cardiovascular sensory inputs can exert quite different effects on PVN presympathetic neurons. For example, arterial baroreceptors inhibit chemoreceptors excite and venous volume receptors inhibit or excite depending which population they are targeting. Such actions are reflected in the differential responses of the cardiovascular target organs (Coote, 2005).

Connections between NTS and the PVN have previously been described, however the final neuronal target of the NTS-PVN projecting was not known until recently (Affleck et al., 2012). We demonstrated afferents from the NTS target at least four types of PVN-associated neurons: presympathetic and putative magnocellular [neuronal nitric oxide synthase (n-NOS)-positive] lying within the PVN and GABA and nNOS-positive neurons surrounding the PVN. A small number of afferent projecting boutons were immunopositive for the vesicular glutamate transporter vGLUT2, suggesting glutamate as neurotransmitter candidate for this pathway (Figure 1B). In addition, The A2 noradrenergic neurons in the NTS that are activated by cardiopulmonary receptors also project to the PVN indicating catecholaminergic a projection could also be a transmitter candidate (Appleyard et al., 2007; Pedrino et al., 2012). Thus in terms of reflex control of cardiovascular regulation we are now in a position to link the sensory inputs to the NTS with specific neuronal targets in the PVN that either directly or indirectly regulate sympathetic activity. We could even go as far as to speculate since, baroreceptors, chemoreceptors and atrial receptors terminate in the region of the NTS, the targeted PVN neurons are representative of the reflex pathway of each of these sensory receptors.

## **Mechanotransduction in the atria**

Atrial volume receptors embedded in the walls of the atria are the mechanosensors signaling plasma volume fluctuations to the NTS via the vagus nerve. Early work on the dog (Kappagoda et al., 1973) clearly demonstrated that stimulation of these receptors produces a reflex reduction in SNA at the level of the kidney. Later studies focusing on the PVN in the rat demonstrated a similar renal SNA effect when the PVN was stimulated with D,L-homocysteic acid (DLH) or the parvocellular neurons of the PVN were selectively lesioned with kainic acid, implying a role for the PVN in this volume reflex response (Lovick et al., 1993; Deering & Coote, 2000). We now know selective stimulation of the right atrial receptors activates a subset of PVN-parvocellular neurons (Pyner et al., 2002).

Atrial stretch related to blood volume return is transduced to an electrical output when mechanosensors undergo mechanical deformation. The mechanosensors comprise channel proteins that when activated results in the generation of the action potentials (Delmas et al., 2001). The channel composition for atrial mechanosensors has not been elucidated. A preliminary study from our lab indicates the transient receptor potential (TRP) family of channels TRPC1 and TRPV4 may be expressed in the endothelium of the right atria of rat heart (Shenton et al., 2012, 2013). This contrasts with the baroreceptor and muscle spindle where Epithelial Na Channel/Degenerin/Acid Sensing Ion Channel (ENaC/DEG/ASIC) families have been reported (Drummond *et al.*, 1998; Simon *et al.*, 2010). Furthermore, we also found the  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels, SK2 and SK4 in this region, although they did not appear to be located in putative mechanosensitive endings (Shenton et al., 2012, 2013). Thus, the volume reflex arc and the resultant sympathetic output needs channel proteins and the anatomical pathways. As such it is therefore necessary to identify the channel proteins that give rise to the mechanosensory properties of the atrial volume receptor and the nature of any neuromodulator present.

## **Neurotransmission**

To return to the PVN, the level of SNA is dependent upon the integration of excitatory-inhibitory activation of the preautonomic neurons by the GABAergic and



glutamatergic interneurons that surround the nucleus (Biancardi et al., 2010). Preautonomic neurons are under tonic inhibition arising from a nitric oxide (NO) mediated GABAergic inhibition at the GABA<sub>A</sub> receptor (Li et al., 2004). While excitatory activation is glutamate dependent via the NMDA receptor, in the anaesthetised rat, tonic glutamate driven excitation in the PVN does not appear to contribute to the prevailing basal level of blood pressure or SNA under normal conditions (Campos & Bergamaschi, 2006). However again in the anaesthetized rat, when SNA is enhanced in HF, kynurenate or the NMDA antagonist AP-5 decreases sympathetic drive, suggesting a glutamate driven excitation on preautonomic PVN neurons now contributes significantly to the level of SNA (Li et al., 2003). The question to ask, is how does this happen?

Accumulating evidence supports the idea that a down regulation of nNOS, the enzyme responsible for the production of constitutively expressed NO generation in concert with altered hypothalamic GABAergic inhibitory and excitatory inputs onto the preautonomic neurons contribute to the exacerbated sympathetic drive in HF (Li et al., 2006). Such that in the PVN of HF rats, GABA<sub>A</sub> receptor density is reduced whereas NMDA receptor density remains unchanged. Thus the down regulation of the GABAergic input combined with a consistent glutamate input within the PVN may be a major candidate that determines resting sympathetic vasomotor tone in HF rats (Carillo et al., 2012). The contribution of other excitatory inputs such as angiotensin II (ANGII) to elevated sympathetic tone in HF must also be considered. Zhu et al., 2004, demonstrated angiotensin II type 1 receptor (AT1-R) blockade in the PVN with losartan reduced renal SNA in HF rats, suggesting that altered AT1-R in the PVN may be involved in elevated sympathetic tone. Also Li-Fan et al., 2006 showed using an in vitro slice preparation that bath application of bicuculline increased the frequency of glutamate-mediated excitatory postsynaptic currents in a PVN-RVLM neuronal projection. The changes in transmitter function probably relates to “oxidant stress” due to an increased production of reactive oxygen species (ROS). Reactive oxygen species generation is a normal by-product of cellular metabolism and is usually kept under tight control by antioxidant enzymes (Zimmerman & Davisson, 2004). Of the many ROS-generating enzymes, activation of the

nicotinamide adenine dinucleotide phosphate (NAD(P)H oxidase or Nox enzymes appear particularly important in HF (Guggilam et al., 2011). Furthermore, an upregulation of the homologue Nox4 in the PVN is associated with sympatho-excitation and impaired cardiac function in the early stages of HF (Infanger et al., 2010). The questions remains: how does the increased Nox-derived ROS contribute to the sympatho-excitation and what triggers the Nox to produce more ROS? It is likely that the production of ROS after activation of the AT1-R and the production of proinflammatory cytokines (PICs) play a major part.

To further demonstrate the crucial role of GABA<sub>A</sub> receptor down regulation, we have shown in the spontaneously hypertensive rat (SHR) protein expression and PVN-preautonomic neuronal expression of the GABA<sub>A</sub>  $\alpha$  1 & 5 subunits is decreased whilst the GluN2A subunit of the NMDA receptor is up regulated. This agrees with Ye et al., (2102) for the SHR who demonstrated not only GluN2A but also GluN2B subunit regulation when compared to age matched Wistar Kyoto controls. We interpret this as the association of GABA<sub>A</sub> and NMDA receptor subunit with PVN-preautonomic neurons providing a mechanism by which the discharge properties of the neuron can be regulated to determine sympathetic output. Therefore, any switch in the subunit of the receptor has the potential to influence the discharge of the presympathetic neurons (Cork et al., 2012,2013). Interestingly, Li et al., (2003) reported an increase in NR1 subunit regulation but no change in NR2 I HF rats. An obvious explanation to the discrepancies relates to animal model selection i.e. HF vs hypertension would argue that the mechanisms producing the sympatho-excitation is related to the prevailing cardiovascular pathology and a such may be used as a discriminator for drug treatments.

## **The Future**

Our wealth of knowledge about the neural control of HF has increased but we still have very little clues to origins or instigator(s) and cellular signaling processes that might be possible drug targets.

## Cellular signaling –Nitric Oxide

While gross changes in neurotransmitter availability and receptor subunit conformational changes can be shown to alter the electrical properties of the preautonomic neuron, the cellular signalling mechanisms leading to the gross changes are lacking. It is probably an alteration of these that give rise to the chronic long-term impact on the overall level of neuronal excitability.

Most research has focused on the cell signaling related to NO. The activity of the nNOS, the enzyme responsible for NO production, is highly controlled and is subject to transcriptional, translational and post-translational regulation, which dictates the specificity of NO signaling and limits NO toxicity (Alderton et al., 2001). Post-translational controls include protein-protein interactions. The protein partners CAPON (carboxy-terminal PDZ ligand-PSD-95/Discs large/zona occludens-1 of nNOS) and PIN (protein inhibitor of nNOS) interact with nNOS specifically through its PDZ and PIN binding domains, respectively. CAPON binding can restrict NO generation by competing with the postsynaptic density protein PSD95 for interaction with nNOS. In HF, nNOS expression decreases via post-translational modification through an ANGII-mediated enhanced expression of PIN. Angiotensin II acting via AT1-R upregulates PIN expression with a concomitant decrease in the expression of nNOS (Figure 2A; Sharma et al., 2013). The action of ANGII at the AT1-R is upregulated in HF. Angiotensin II stimulation of the AT1-R potentiates neuronal excitability and this effect is constrained by a NO-GABAergic feedback system that suppresses AT1-R activation (Li et al., 2003). In HF, the inhibitory modulation of the AT1-R is reduced and this may upregulate PIN expression in the PVN inhibiting nNOS activity the subsequent production of NO and removal of tonic neuronal inhibition. The role of PIN on glutamate and GABA is indirect via its actions on nNOS.

In the brain NO biosynthesis depends on the availability of the NOS cofactors, for example (6R)-5,6,7,8-tetrahydrobiopterin (BH4) as well as NAD(P)H as an electron source. Homodimerisation and cofactor binding are critical for the enzymatic activity of nNOS. The dimerization of nNOS is promoted by heme incorporation and BH4 is required to stabilize the nNOS dimer once it is formed (Alderton et al., 2001). Neuronal nitric oxide activation without proper BH4 binding uncouples normal

electron transfer to produce superoxide. In HF, BH4 bioavailability is known to be impaired in endothelial cells but there is no evidence for reduced BH4 bioavailability in the PVN (Schmidt & Alp, 2007). Thus the role of NOS cofactors in nNOS regulation in HF remains to be elucidated.

### **Cytokines and superoxide**

In HF, apart from activation of neurohormones there is also activation of PICs such as tumor necrosis factor (TNF), interleukin (IL)-1 $\beta$  and IL-6 in the PVN and the level of circulating cytokines correlates with deteriorating HF (Francis et al., 2004).

Following an acute myocardial infarction (MI) elevated PICs are transported into the hypothalamus and brainstem via the circumventricular organs and within minutes TNF is observed in the brain (Francis et al., 2004). Circulating ANGII and PICs are also major activators of microglial cells, astrocytes and macrophages, which consequently produce more PICs (Shi et al., 2013). The PICs are distributed particularly in the PVN and medulla, sites that regulate SNA to various cardiovascular and fluid homeostatic organ systems (Guggilam et al., 2011).

Intracerebroventricular administration of ANGII increases the production of reactive ROS (Zimmerman et al., 2002). Angiotensin II type 1 receptor blockade in the PVN and RVLM attenuates ROS generation contributing to decreased SNA in HF (Han et al., 2007). In addition, previous investigations have shown that in HF, the increase in TNF in the PVN is associated with elevated expression of NAD(P)H oxidase subunits, the primary source of super oxide anions ( $O_2^{\cdot -}$ ) and intimately involves the actions of the renin-angiotensin system (Guggilam et al., 2011). Moreover, blockade of PVN  $O_2^{\cdot -}$  completely abolishes the increased SNA observed in HF (Figure 2B; Han et al., 2007).

### **Neuro-glial signalling**

One final aspect to consider is the bidirectional neuro-glial signaling in neurohumoral regulation in the PVN. A number of mechanisms have been described and succinctly summarized in a recent review by Stern & Filosa (2013). Microglia respond to injury and pathogen infection in the brain, turning into an activated pro-inflammatory state. Active microglia release a variety of pro-inflammatory and also neuroactive

substances, including cytokines, chemokines, ROS and NO, among others leading to exacerbated neuronal activity and ultimately neuronal cell death. Importantly, there is a growing body of evidence suggesting cardiovascular-related signals in particular ANGII can lead to microglial activation (Stern & Filosa, 2013), contributing in turn to exacerbated neurohumoral activation in disease conditions such as HF, hypertension and diabetes (Guggilam et al., 2011).

## **Conclusion**

Despite a wealth of studies over the last couple of decades providing major insights into autonomic control, we still have significant gaps in our knowledge. It would appear there are some common neuroactive substances, PICs, ROS and NO that affect cellular and molecular system. We can describe the contribution each makes individually to disrupt neuronal excitability but have very little insight about interaction and what seems crucially important the sequence of events and a trigger. The importance of this probably lies within devising new treatments that might show distinct pharmacological characteristics depending on the stage of progression of the HF.

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## Figure Legends

### Figure 1

Schematics to show the components of the volume reflex arc. Volume receptors in the heart (A) provide the afferent signals to the NTS, which are then conducted to the PVN. Direct and indirect efferent projections influence sympathetic outflow at the level of the SPN. At least four ascending pathways (B) from the NTS target PVN neurons that are associated with cardiovascular control: (1) spinally projecting neurons. (2) nNOS-containing magnocellular neurons. (3) GABAergic interneurons that contact spinally projecting neurons. (4) nNOS-containing interneurons bordering the PVN. A provided by G Watson and B modified from Affleck et al., 2012.

### Figure 2

Schematic summaries proposing how alteration of signaling pathways can lead to abnormal sympathoexcitation. Postranslational modification of nNOS alters the regulation of the PVN in HF. Over activation of the AT1 receptor leads to over-expression of CAPON and PIN. CAPON and PIN interfere with nNOS activation reducing NO production in the PVN causing an increase in SNA. Ischaemic injury (B) releases PICs. The PICs signal to the PVN causing the release of further PICs and neurohormones leading to the imbalance of superoxide and NO and sympathoexcitation. A modified from Sharma et al., 2013 and B modified from Guggilam et al., 2011.

Figure 1

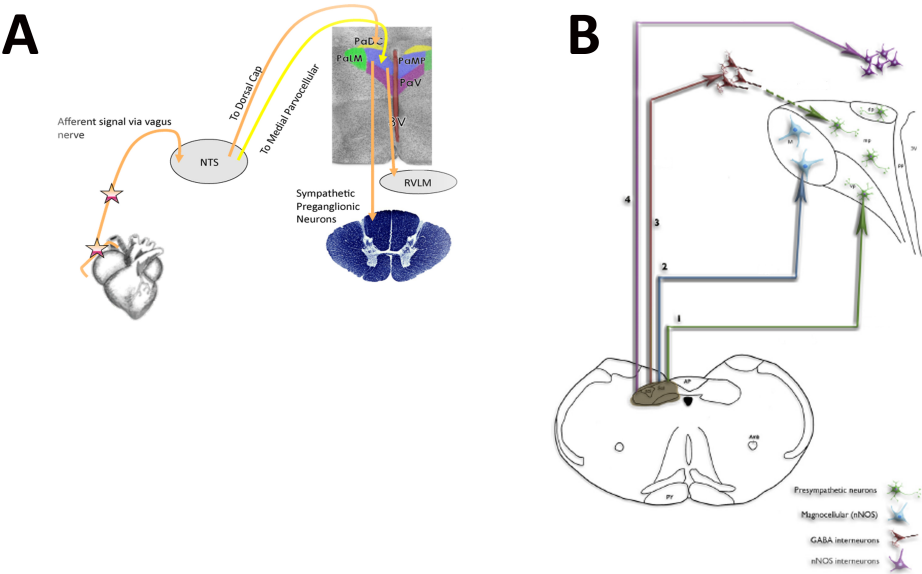


Figure 2

